

AMENDMENTS TO THE SPECIFICATION

Please amend the Abstract as follows:

ABSTRACT

A reagent system is provided for substantially lysing red blood cells in a whole blood sample prior to leukocyte analysis, ~~the reagent which~~ system includes~~[[:]]~~ a first reagent for substantially lysing the red blood cells in the whole blood sample, and a second reagent for quenching the activity of the first reagent, ~~wherein the second reagent includes a base and has a pH value of about 8 to 12. A final acidic media, ranging from about pH 4 to about 6, is used to stabilize the white blood cells and continuously remove red blood cell fragments.~~ The first reagent is formulated to include~~[[: a]]~~ an autoclaved saponin compound; and an acid selected from the group consisting of a halogenated carboxylic acid~~[[s]]~~, a phosphoric acid ~~or~~ and a combination~~[[s]]~~ thereof. The second reagent includes a base and has a pH value of about 8 to 12. and optionally a surfactant. In addition Also provided is a method of lysing the red blood cells and stabilizing white blood cells present in a sample of whole blood, ~~includes the steps of: combining a predetermined portion of the sample of whole blood with a predetermined portion of a first reagent for substantially lysing the red blood cells and stabilizing white blood cells in the whole blood sample, wherein the first reagent includes: a saponin compound; and an acid; and quenching the lysing action of said first reagent by the addition of a predetermined portion of a second reagent, wherein the second reagent includes a base and has a pH value of about 8 to about 12 to give a solution containing substantially lysed red blood cells, leukocytes and a pH value of about 3 to about 6.~~

Please amend the paragraph bridging pages 1 and 2 as follows:

The subject matter of the present disclosure is generally directed to a reagent system for substantially lysing red blood cells in a whole blood sample prior to leukocyte analysis. In one illustrative embodiment, the reagent system includes[[,]] a first reagent for substantially lysing the red blood cells in the whole blood sample, and a second reagent for quenching the activity of the first reagent, wherein the second reagent includes a base and has a pH value of about 8 to 12. A final acidic ~~media~~ medium, ranging from about pH [[4]] 3 to about 6, preferably from about 4 to about 5, is used to stabilize the white blood cells and continuously remove red blood cell fragments. The first reagent is formulated to include: a saponin compound; an acid, preferably selected from halogenated carboxylic acids, phosphoric acid or combinations of these and similar compounds that should be known to one of skill in the art. Optionally the first reagent may further include a surfactant preferably selected from non-ionic surfactants, cationic surfactants and combinations of these and similar compounds that should be known to one of skill in the art. In one specific and illustrative embodiment, the surfactant is selected from ethoxylated decylalcohols, ethoxylated and propoxylated linear (C8 – C10) aliphatic alcohols, and combinations of these and similar compounds that should be known to one of skill in the art. It should be appreciated that the saponin compound is preferably selected from the group including saponin; heat-treated saponin, saponin modified by heating in the presence of a halogenated carboxylic acid and combinations of these and similar compounds that should be known to one of skill in the art.

On page 2, lines 8-27, please amend the paragraph as follows:

Another illustrative embodiment of the claimed subject matter includes a reagent system formulated to include: a reagent for lysing red blood cells; and a quench; such that the system is substantially free of compounds including: i. dye; ii. a combination of saponin and carboxylic acid; iii. an acid selected from formic acid, acetic acid and mixtures thereof; iv. a combination of saponin and sulphonic acid; v. a cross-linking agent such as an aldehyde; vi. an alkali metal salt of an alkyl sulfate anionic surfactant; vii. an ethoxylated long chain amine; and viii. combinations thereof. A final acidic ~~media~~ medium, ranging from about pH ~~[[4]]~~ 3 to about 6, preferably from about 4 to about 5, is used to stabilize the white blood cells and continuously remove red blood cell fragments. Preferably the illustrative reagent for lysing red blood cells includes a saponin compound and an acid. The saponin compound can be selected from saponin; heat-treated saponin, saponin modified by heating in the presence of a halogenated carboxylic acid and combinations of these and similar compounds that should be known to one of skill in the art. The acid portion of the reagent system is selected from halogenated carboxylic acids, phosphoric acid or combinations of these and similar compounds that should be known to one of skill in the art. The reagent for lysing red blood cells may further ~~includes~~ include a surfactant. The surfactant can be selected from non-ionic surfactants, cationic surfactants and combinations thereof and preferably the surfactant is selected from ethoxylated decylalcohols, ethoxylated and propoxylated linear (C8 – C10) aliphatic alcohols, and combinations of these and similar such compounds.

On page 3, line 21, before the section for the “Description of Illustrative Embodiments”, please add the following heading and paragraphs:

BRIEF DESCRIPTION OF THE DRAWING

Features of the present invention as well as a preferred mode of use, further objectives, and advantages thereof, will best be understood by reference to the following detailed description of an illustrative embodiment when read in conjunction with the accompanying drawing, wherein:

FIG. 1 illustrates comparison of lytic stability of the red blood cell lyse solutions at 40°C in accordance with the present invention.

On page 3, lines 23-28, please amend the paragraph as follows:

The red blood cell lyse agent of the claimed subject matter preferably includes first component for lysing red blood cell components of a blood sample, a second component that is an acid, and an optional third component that functions as a surfactant. The combination of reagents is designed to achieve a final acidic ~~media~~ medium, ranging from about pH ~~[[4]]~~ 3 to about 6, preferably from about 4 to about 5, is used to stabilize the white blood cells and continuously remove red blood cell fragments.

On page 5, lines 17-22, please amend the paragraph as follows:

It is seen from the above that promptly after contacting the blood sample with the red blood cell lyse, it is desirable to quench a substantial portion of the remaining white blood cell fraction of the blood. This is accomplished by raising the osmolality to isotonicity and bringing the pH to a value of approximately 4.5. The quench is used to achieve a final acidic ~~media~~ medium, ranging from about pH ~~[[4]]~~ 3 to about 6, preferably from about 4 to about 5, which is designed to stabilize the white blood cells and continuously remove red blood cell fragments.

On page 6, lines 10-13, please amend the paragraph as follows:

The amount of the quench utilized should result in a final solution having a pH value of about 3.0 to about ~~7.0~~ 6.0 and preferably from about 4 to about 5. That is to say a slightly acid solution is produced to stabilize the leukocytes and preserve the differentiation of leukocytes.

On page 13, lines 7-27, please amend the paragraph as follows:

In view of the above disclosure, one of ordinary skill in the art should understand and appreciate that one illustrative embodiment of the claimed subject matter includes a reagent system for substantially lysing red blood cells in a whole blood sample prior to leukocyte analysis. In one such illustrative embodiment, the reagent system includes[[,]] a first reagent for substantially lysing the red blood cells in the whole blood sample, and a second reagent for quenching the activity of the first reagent, wherein the second reagent includes a base and has a pH value of about 8 to 12. A final acidic ~~media~~ medium, ranging from about pH [[4]] 3 to about 6, preferably from about 4 to about 5, is used to stabilize the white blood cells and continuously remove red blood cell fragments. The first reagent is formulated to include: a saponin compound; an acid, preferably selected from halogenated carboxylic acids, phosphoric acid or combinations of these and similar compounds that should be known to one of skill in the art. Optionally the first reagent may further include a surfactant preferably selected from non-ionic surfactants, cationic surfactants and combinations of these and similar compounds that should be known to one of skill in the art. In one specific and illustrative embodiment, the surfactant is selected from ethoxylated decylalcohols, ethoxylated and propoxylated linear (C8 – C10) aliphatic alcohols, and combinations of these and similar compounds that should be known to one of skill in the art. It should be

appreciated that the saponin compound is preferably selected from the group including saponin; heat-treated saponin, saponin modified by heating in the presence of a halogenated carboxylic acid and combinations of these and similar compounds that should be known to one of skill in the art.